The University of Chicago

A COMPARISON OF THE DESCENDANTS OF LEAD-POISONED MALE GUINEA-PIGS WITH THOSE FROM UNTREATED ANIMALS OF THE SAME CLOSELY INBRED STRAINS

A PART OF THE DISSERTATION SUBMITTED
TO THE GRADUATE FACULTY IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
EDWARD CECIL COLIN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 60, No. 4, 1931

The University of Chicago

A COMPARISON OF THE DESCENDANTS OF LEAD-POISONED MALE GUINEA-PIGS WITH THOSE FROM UNTREATED ANIMALS OF THE SAME CLOSELY INBRED STRAINS

A PART OF THE DISSERTATION SUBMITTED
TO THE GRADUATE FACULTY IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
EDWARD CECIL COLIN

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 60, No. 4, 1931

R. UNIV.
BIBLIOTHEEK
LEIDEN

Reprinted from THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 60, No. 4, November, 1931

A COMPARISON OF THE DESCENDANTS OF LEAD-POISONED MALE GUINEA-PIGS WITH THOSE FROM UNTREATED ANIMALS OF THE SAME CLOSELY INBRED STRAINS

E. C. COLIN
Department of Zoölogy, The University of Chicago

SIX FIGURES

INTRODUCTION

The present experiments were undertaken as a contribution to the question as to whether or not it is possible to injure or alter the offspring of mammals by poisoning the sire with lead. The inquiry is naturally included under the general problem of the direct effect of environmental agents on the germ cells. It is not a question of the inheritance of acquired characters in the strict Lamarckian sense, for obviously the germ cells are simultaneously exposed to the same conditions as the soma, and any effect produced on the offspring might reasonably be attributed to the direct action of the agent on the germ cells.

In the interest of economy of space, no review of the extensive literature on the general problem with agents other than lead will be presented. It may be recalled, however, that numerous experiments have been performed by various investigators, using a wide variety of chemical and physical agents, such as alcohol, lead, manganese, immune sera, high temperatures, and radiations. In many cases positive effects have been claimed, often to be later cast in doubt by negative results of other workers or by criticism of control or technique. No agent save radiations may be said to have yielded a satisfactory series of positive results in the hands of a

number of workers. As the matter stands to-day, it is generally regarded by critical students as still an open question whether or not such agents other than radiations, administered to the male, result in positive effects on the offspring.

The question of the effect of lead is of special interest, on account of its relation to human affairs. Lead poisoning is the most common type of industrial poisoning. The possible injurious effects of lead upon the offspring of lead-poisoned men have for years received the attention of the medical profession. It has been widely accepted by writers on lead poisoning that a man's children may be injuriously affected as a result of the lead absorbed by the father.

The direct evidence in support of this opinion consists in certain clinical and statistical studies made chiefly in France. One of the earliest of these, and one often quoted, is that of Constantin Paul, interne in the Hospital of Paris ('60). Paul obtained his information by questioning the wives of seven lead workers who had shown symptoms of plumbism. The seven wives had thirty-two pregnancies, eleven ending in abortion and one in stillbirth. Of the twenty children born alive, eight died in the first year, four in the second, five in the third, and one after the third, leaving only two alive of the thirty-two pregnancies. No control studies were made of the wives of workers in other lines than lead. Paul does, however, give the following statistics for the city of Paris as a whole. In 1856 there were 40,493 births, of which 37,697 were living and 2796 (6.90 per cent) were dead. Of the 37,697 living infants, 5596 died during the first year, 2042 during the second year, and 1006 during the third year, making a total of 8644 for the first three years. No statistics on abortions for the city as a whole were, of course, obtainable.

Adding stillbirths and deaths during the first three years, one obtains 11,440, or 28.3 per cent of all births. Compared with this there were from the seven wives of lead workers eighteen stillbirths and deaths during the first three years, or 85.7 per cent of all births. Great as the difference is between these two percentages, there is nevertheless some doubt

as to its significance in the absence of any assurance that the parents in the lead-poisoned series represent a fair sample of the population of Paris as a whole.

There seems also to be a qualitative objection to the data as presented by Paul, which has apparently not been noted by anyone else heretofore, namely, that two of the seven wives had worked in the foundry where their husbands presumably contracted lead poisoning, although, Paul states, without the women themselves becoming poisoned. Under such circumstances we are therefore left to wonder whether or not the possibility of direct poisoning of the mothers was excluded in Paul's data. In fairness to Paul it should be stated that he did not regard the effect of paternal plumbism on the offspring as demonstrated by his data; he merely regarded such an effect as probable.

The data regarded by Hamilton ('25) as "much the most convincing that have been presented to show the influence of paternal plumbism on the human offspring" were statistics assembled by Deneufbourg ('05). He obtained his data from the archives of the Bandelocque Obstetrical Clinic of Paris. In the histories of 151 women, 'free from lead poisoning,' there were 442 pregnancies by workmen exposed to lead. These pregnancies terminated as follows:

66 by abortion
51 by premature birth at the seventh month
45 by premature birth at the eighth month
147 by premature birth during the ninth month
133 by birth at term

In forty-seven cases from the last four groups the fetus was dead and macerated, leaving 329 born alive at or before term. Of those living, eighty-three died during the first year.

Similar data were obtained where the mother alone was poisoned and where both parents were poisoned. Arranged in tabular form, his data are as follows:

	Number of pregnancies	*Abortions*	*Still-births*	*Abortions and still-births, per cent*	*Born living*	*Died first year*	*Died first year, per cent*
Father leaded,	442	66	47	25.5	329	83	25.2
Mother leaded,	134	17	6	17.0	111	29	26.1
Both leaded,	23	4	4	35.0	15	5	33.3

As Hamilton justly remarks, the second and third groups are too small to be taken with much seriousness.

That Deneufbourg does not consider that an effect of paternal plumbism has been demonstrated by these data is evidenced by the fact that, in his discussion of the cause of the effects, he mentions two possibilities: 1) the possible injury of the sperm by the lead and, 2) the possibility that the wife may herself have been poisoned more or less, either by working in the shop with her husband or in caring for his clothing. No control studies were made by Deneufbourg.

In discussing the causes of death of the children the author remarks upon the number dying with nervous manifestations, convulsions, etc.

Additional statistical studies as well as a number of more or less isolated cases purporting to show similar effects are reported in the French and German medical literature. Citations for these are given by Hamilton ('25) and by Aub, Fairhall, Minot, and Reznikoff ('26); they are therefore not repeated here.

On the other hand, in England Legge and Goadby ('12) find very little evidence for the effect of paternal plumbism on the offspring. They write as follows:

> In the Report of the Committee on the Use of Lead in the Potteries, some inquiry was made with regard to the possible association of lead absorption on the male side as a predisposing cause of infant mortality and premature birth. The tables given are not very conclusive, and from our own observations there seems to be very little evidence for supposing that a male lead worker is less likely to beget children, or that his children are more likely to be unhealthy than those of men working in any other industrial process. We are here speaking of the effect of lead under the conditions of its general use in this country now. In the absence of any precautions whatever as to the daily absorption of dangerous dust, the effect on the offspring, even in the case of male lead workers, may well be evident, as has been shown by Chyzer in the manufacture of pottery as a home industry in Hungary.

The qualification contained in the last sentence loses much of its weight when it is noted that the work in lead in Hungary

is often carried on in the home in the living and sleeping rooms, the entire family being exposed to the poisonous dust (Oliver, '11). Some of the children have, in fact, been observed to show the typical effects of plumbism (Rennert, '81).

Turning now from these inconclusive human data to the results of animal experimentation, one finds that there have been at least two experiments with mammals along the same lines as the present ones. The first of these reported is that of Cole and Bachhuber ('14). Lead acetate, mixed with milk sugar to increase the bulk and the ease of handling, was fed in gelatin capsules to males of the rabbit as well as to the fowl. The method of double matings was employed, i.e., a female of a certain type was bred to two males during the same period. The males were of such a genetic constitution that the young of each could be identified by color or other characters. With the rabbits two series of experiments were performed. In series I a normal homozygous Dutch-marked male and a poisoned albino male were bred to a number of normal albino females. In series II an albino male was used as the normal or control, while the previous control male (Dutch-marked) was treated with lead. The results were as follows:

SERIES	NORMAL MALES	POISONED MALES	ALBINO YOUNG		PIGMENTED YOUNG		AVERAGE WEIGHT AT BIRTH	
				Per cent dead in 4 days		Per cent dead in 4 days	Albino	Pigmented
I	D-m 20.2	Albino 26.8	84	47.7	90	18.9	49.8	54.7
II	Albino 21.4	D-m 20.2	27	29.2	147	34.2	59.0	49.1

In series I there were nine successful double matings and thirteen unsuccessful double matings. In series II there were five successful double matings and fifteen unsuccessful double matings. Both albino males were considerably larger than the Dutch-marked male, the former varying around 2900 grams, while the latter averaged only about 2100 grams.

From these results the authors state that it seems legitimate to conclude that the offspring produced by male rabbits which

have been poisoned by the ingestion of lead acetate into the alimentary tract have a lower vitality and are distinctly smaller in average size than normal offspring of unpoisoned males.

With fowls similar experiments were performed. The color, comb, and toe characteristics could be used in distinguishing the chicks of the respective cocks. The results were as follows:

From four White Leghorn hens bred to poisoned Leghorn cock:

Total eggs	*Infertile eggs*	*Per cent infertile*	*Dead embryos*	*Per cent fertile eggs dead*	*Chicks hatched*	*Dead in 3 weeks*	*Per cent dead in 3 weeks*
174	47	27.0	35	27.5	92	13	14.1

From three White Leghorn hens bred to normal Houdan cock:

Total eggs	*Infertile eggs*	*Per cent infertile*	*Dead embryos*	*Per cent fertile eggs dead*	*Chicks hatched*	*Dead in 3 weeks*	*Per cent dead in 3 weeks*
111	47	42.3	11	17.2	53	2	3.7

From three White Leghorn hens bred on alternate days to poisoned Leghorn cock and to normal Houdan cock:

Total eggs	*Infertile eggs*	*Dead embryos*	*Identified*		*Chicks hatched*		*Per cent Leghorn dead in 3 weeks*	*Per cent cross-bred dead in 3 weeks*
			Leghorn	*Cross-bred*	*Leghorn*	*Cross-bred*		
109	46	17	9	1	31	15	16.1	0.0

The results are interpreted by the authors as indicating that in fowls also, poisoning of the male parent with lead results in offspring of a distinctly lower average vitality.

The second investigation with mammals was conducted by Weller ('15). The animals used by him were guinea-pigs. Commercial white lead mixed with linseed oil and corn starch in the form of a paste was given by mouth in no. 5 gelatin capsules. The dosage varied between 0.006 gram and 0.082 gram administered every other day. Care was taken that there should be no marked loss in weight, and any such drop in weight was the occasion for reducing the dosage, increasing the interval between doses, or temporarily suspending treatment.

In some cases there were sudden reductions in weight such that no lead could be administered for several weeks. In a few others epileptiform convulsions were induced even when there had been no loss in weight, and following the convulsive attacks such pigs were so ill that no lead was given for several days. With these exceptions lead was administered throughout the greater part of the period covered by the experiment. The experiment extended over a period of eighteen months. The maximum length of time to which any animal was subjected to treatment is not stated, but in a series of seven animals selected by the author as representative, the duration ranged from three and one-half to twelve months.

In one series a system of alternate matings was carried on in which normal females were alternately bred to treated males and to normal males.

Summarized, the results of all the experiments are as follows:

	Matings	*Offspring*	*Average birth weight, grams*	*Stillborn*	*Died first week*
Normal ♂ / Normal ♀	32	58	81.5	3	2
Leaded ♂ / Normal ♀	34	65	66.3	3	9
Normal ♂ / Leaded ♀	27	47	69.3	8	0

In most cases no lead was given to females during the nursing period. This, thinks Weller, accounts for the higher birth weights of offspring from treated females than from treated males.

In only two instances were malformations observed in the offspring of the treated animals—whether from treated males or treated females is not stated. Two pigs showed a slight malformation of the upper jaw with deformed teeth. The author suggests that this may have been the result of some nutritional disturbance.

The stillborn offspring from lead-poisoned females were usually premature, and often showed by maceration of the skin that they had been dead for some time before expulsion.

In general, no marked reduction in fertility was found, although in several instances lead-poisoned males failed for three or four months to impregnate any of the females with which they were running. The average number of offspring per mating in this group was practically the same as in the group of normal pigs. Only one of the lead-poisoned males proved absolutely sterile. Likewise in the female there was no loss of fertility.

The author states that those offspring of a lead-poisoned parent which are decidedly below the average weight at birth remain permanently underweight as compared with normal pigs of the same age. The only data presented on this point are the weight curves of two animals from birth to about twenty-five days of age. One is from a lead-poisoned mother and the other from normal parents. The former at birth was about 40 grams under the latter in weight. The curves run practically parallel for the time indicated.

Observations were reported for only the first filial generation, and from the results obtained the conclusion was drawn that in chronic lead poisoning in male guinea-pigs there is a definite blastophthoric effect, manifested in some instances by sterility without loss of sexual activity, by a reduction of approximately 20 per cent in the average birth weight, by an increased number of deaths in the first week of life, and by a general retardation in development such that the offspring of a lead-poisoned male remain permanently underweight. No data are presented in support of the statement as to the permanent effect of lead on the weight of offspring of a lead-poisoned male.

In a later report Weller ('16) describes histological studies of the testes of guinea-pigs used in the lead experiments. In the majority of cases examined no histological differences could be detected between the testes of the lead-poisoned guinea-pigs and those of the controls, but in a 'limited number' of instances in which the male guinea-pigs became sterile during the administration of lead, histological examination of the testes showed a complete aspermatogenesis

with marked atrophy and vacuolar degeneration of the germinal epithelium. "There is an attempt at cell division which results in multinuclear giant cells, evidently comparable to spermatocytes, but division of the protoplasm appears to lag behind that of the nuclei and spermatids and spermatozoa are not produced."

A critical analysis of the work of Cole and Bachhuber and of Weller is offered in the Discussion (p. 479).

Several experimenters have treated insects with lead and other chemical agents. Mann ('23), using what she described as a 'stable' stock of Drosophila melanogaster, failed to obtain increased mutability or tendency to abnormality. In addition to lead acetate, larvae were treated with arsenic, quinine, morphine, strychnine, copper sulphate, lithium carbonate, methylene blue, the fumes of ethyl alcohol and methyl alcohol, high temperatures, low temperatures, and the accumulation of gases of fermentation of the culture medium. In none of these were positive effects obtained.

In the experiments with lead the author found that mechanical difficulties made the use of lead acetate with Drosophila unsatisfactory. Soluble lead acetate caused the banana protein to coagulate and drop to the bottom of the medium. She therefore placed the flies in culture bottles containing the usual food, which were then filled with masses of paper soaked in lead-acetate solution. From such cultures the broods were described as large and normal, in all 3772 flies being examined. In five successive generations so treated with lead acetate no mutations occurred nor was somatic abnormality increased.

Muller ('28) reports the use of five different agents, including lead acetate, with Drosophila melanogaster. Approximately 500 cultures of the C1 type, made up especially for the detection of lethals, were used with each agent, and the same number (500) were used as controls. In the lead experiment, 1 per cent of lead acetate was included in the food, on which the flies were placed from the egg stage through maturity. Muller mentions no difficulty with the coagulability of the protein in the culture medium used by him.

Another treatment involved a similar protracted feeding on arsenic trioxide, in a semilethal concentration (0.015 per cent of the food). In a third treatment semilethal doses of manganese chloride (0.62 per cent and 0.31 per cent of the food) were administered in like manner.

In the experiments with lead, arsenic, and manganese, combined, there was only one lethal (and no other) mutation observed. The lethal was in a manganese culture. The controls yielded two lethals.

Harrison and Garrett ('25) fed three species of geometrid moths for several generations with vegetation contaminated with lead nitrate or with manganous sulphate, in order to test the effects of these agents in the production of melanism. Melanism is said to occur naturally in moths in certain smoky industrial districts in England, and the suggestion has been made that this is due to the ingestion by the larvae of vegetation contaminated with lead and other chemicals.

Positive results were obtained both with lead and with manganese. Relatively large numbers of melanic forms appeared in the treated series and none in the controls. When tested by appropriate breeding methods, the melanic forms all behaved as simple mendelian recessives, with the exception of one which was dominant in the first cross.

The one dominant appeared in Tephrosia crepuscularia as a single black female in a brood of twenty-three moths reared on contaminated bushes gathered by the roadside. It was paired with an ordinary male and an F2 generation of twenty-one insects emerged, all of which were black. It was therefore regarded as a mendelian dominant, as in the case of the similar form which is found wild. No further experiments were performed with this species.

In another species, Selenia bilunaria, numbers of melanic forms appeared. For example, in one series treated with lead the P1's were normal, but in two broods of F1's (also treated) the ratios of normals to melanics were 26:1 and 29:2, respectively. In another series with the same species, using manganese, there appeared in a brood of F1's twenty moths,

eight of which were black and all the rest in varying degrees 'melanochric.' These were tested in appropriate matings and the results indicated that the black individuals were simple homozygous recessives and the melanochrics were heterozygotes.

In the third species, Tephrosia bistortata, melanism likewise appeared in two distinct series. When tested it behaved as a simple mendelian recessive.

In their interpretation of the results the authors incline toward a genuine Lamarckian explanation, although they regard the question as to whether the effect was truly Lamarckian or merely parallel induction as academic.

Harrison ('27) repeated the experiments, using one of the species previously employed (Selenia bilunaria), in order to determine whether the metal or the acid radical was responsible for the effects. He therefore used manganese chloride instead of the sulphate. Positive results were obtained similar to those obtained in the previous experiments.

The author here denies that the effect is of the Lamarckian type, but rather considers that it has been produced by the direct action of the metallic compound on the germ plasm.

Among all the experiments in which external agents have been employed in treating the organisms themselves, these on melanism stand out as unique in two respects: 1) in the large numbers of visible mutations induced, amounting in one experiment with manganese, mentioned above, to 100 per cent either in the homozygous or in the heterozygous condition, 2) in the remarkable specificity of effect. No mutations other than melanism were reported, while melanism was found in at least five independent series of experiments involving two distinct genera of moths. With radiations there is never an approach to 100 per cent visible mutations and those mutations that do occur seem practically always to arise first in the heterozygous condition. With radiations, also, the mutations appear in great variety, with no seeming order or predictability. It would seem, therefore, that there must be some different principle involved in the origin of melanism in moths than in the origin of mutations following radiation.

In this connection it is interesting to note that Mansbridge ('27) obtained melanic forms in Selenia bilunaria without treatment, merely by selecting the darkest individuals in each generation. A wild female was captured and four generations were bred. The F4 generation is described as "remarkable for the fact that five specimens in a total of about thirty showed a strong tendency to melanism and two others were almost as black." Fertile ova were obtained from them, but as no food was available for the young larvae, they died. Likewise, Cockayne ('27) reports the appearance of melanics, resembling those obtained by Harrison, from untreated Selenia bilunaria. There would consequently appear to be some question as to whether or not treatment was the cause of mutation in the experiments of Harrison and Garrett.

Sonneborn ('30) studied the effects of lead on Stenostomum incaudatum, a turbellarian flatworm that reproduces by transverse fission. For a number of asexual generations it was immersed in solutions of lead acetate. All lines became abnormal and unless removed from the solution died. Of those removed, many survived. Their abnormalities either disappeared by regulatory processes or were transmitted for one or more generations. Within a few generations under control conditions, all the abnormals had died out and the normals produced by them had given rise to a completely normal population, except in two instances in which there arose two different kinds of double individuals. The doubling persisted from generation to generation. The double individuals were like those frequently arising from old declining parents, untreated, except that in the latter case the doubles never gave rise to double progeny, but always died soon. The author regards the origin of the double individuals as one of dedifferentiation and cessation of growth followed by the establishment of two new axes precisely as is called for by the theory of twinning set forth by Newman ('21).

In order to put the matter of the possible effects of paternal plumbism upon the offspring of mammals to a further, and if possible still more critical, test, experiments with lead were

begun by the writer in the summer of 1926, following the suggestion of Dr. Sewall Wright. To insure the genetic homogeneity of the experimental animals, there were in March, 1927, made available two highly inbred families of guinea-pigs which had the previous year been brought from the U. S. Bureau of Animal Industry at Washington. Using these inbred animals, the experiments were carried on continuously up to the summer of 1930. The writer hereby expresses to Doctor Wright his sincere thanks for suggesting the problem and for furnishing the experimental animals, which are so admirably adapted to this type of experiment, and for his valuable advice and counsel throughout the work.

MATERIAL AND METHODS

History of the guinea-pig stock

Since the use of homogeneous stock of known genetic constitution is the first requisite in any experiment in which the effects of environmental agents are to be studied, the history of the animals used will be given in some detail. All the experiments, with the exception of a few preliminary ones, have been done with two highly inbred strains or 'families' (family 2 and family 13). The origin and history up to 1925 of these families have been reported in full by Wright ('22 a, '22 b) and by Wright and Eaton ('29). In brief, the families were started by the U. S. Bureau of Animal Industry in 1906 as a part of an experiment for the purpose of studying the effects of inbreeding when continued for successive generations. The pedigrees of families 2 and 13 from the fifteenth and twentieth generations, respectively, up to June 30, 1930, are shown as figures 1 and 2. The matings numbered 0 (not shown in the figures) were between males and females selected from the general breeding stock of the Bureau—a stock that had already been maintained for twelve years without outcross. Matings numbered 1 were between brothers and sisters from the foundation matings of each family and thus produced a progeny with one generation of inbreeding back of it. The matings of all later generations were between brothers

and sisters, the generation number thus always referring to the number of successive brother-sister matings in the ancestry of the progeny produced.

The animals were mated at the time of weaning, when thirty-three days of age—thirty days since they were brought to Chicago—and in order to avoid the possibility of mistakes, this was still further limited in nearly all cases to litter

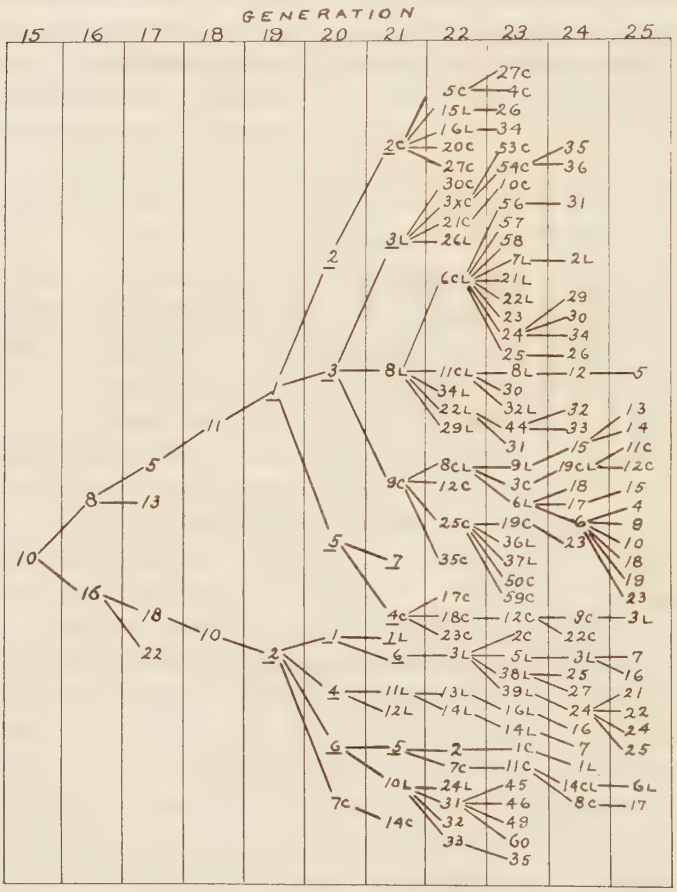

Fig. 1 Pedigree of family 2, Chicago branch, as of June 30, 1930, from the fifteenth to the twenty-fifth generation. Each number in the figure represents a mating between a brother and a sister. *C*, control; *L*, treated; *CL*, used first as control and later as treated. Numbers underlined represent the fifteen matings brought from Washington to Chicago in 1926.

mates. A single pair has been kept in each pen with the exception of cases in the early generations and certain cases, hereinafter referred to, since the writer began his experiments.

It will be noted that all the animals used in family 2 come from one pair in the fifteenth generation, and all those in

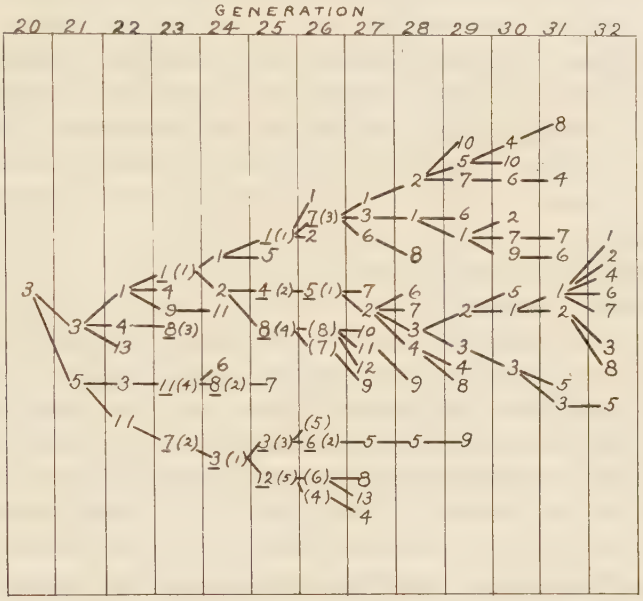

Fig. 2 Pedigree of family 13, Chicago branch, as of June 30, 1930, from the twentieth to the thirty-second generation. Each number in the figure represents a mating between a brother and a sister. Numbers in parentheses are Chicago numbers. Beginning with the twenty-seventh generation, all matings bear Chicago numbers only. Numbers underlined represent the fourteen matings brought from Washington to Chicago in 1926.

family 13 come from one pair in the twentieth generation, and therefore have fifteen and twenty generations, respectively, of common brother-sister ancestry. It is well known from experience that inbreeding tends automatically to bring about a homogeneous condition in a population. Theoretically there seems no doubt that this is accomplished by reducing the heterozygous condition to a homozygous one. The theoretical

reduction under brother-sister mating was calculated by Fish ('14) and Pearl ('14) by generations. As shown by Wright ('31), the rate per generation is about 19.1 per cent. On this basis, in the absence of disturbing factors, the Chicago branch of family 2 should be about 97 per cent homozygous, and the Chicago branch of family 13 about 99 per cent homozygous, with respect to factors heterozygous in the foundation stock. All the studies that have been applied to the two families agree in general with this theoretical expectancy (Wright, '22 a, '22 b, and, especially, Wright and Eaton, '29). The individuals in each family are extraordinarily homogeneous, yet the two families differ markedly from one another in many respects, as well as from other families of guinea-pigs.

The absence of any decline in vigor or signs of degeneration in either family in recent years indicates that a close approach to homozygosis has been attained. Another line of evidence comes from the results of transplantation of tissues (thyroid, pancreas, etc.) between animals of the same family and between those of different families (Loeb and Wright, '27). Transplantations between animals of the same inbred families (largely family 2 and family 13) gave results approximating those of autotransplants, even when there were many generations of separate ancestry. The results were almost perfectly those of autotransplants where there were more than eleven generations of common ancestry. In transplants between families, on the other hand, the grafted tissues were destroyed.

Characteristics of family 2 and family 13

Compared with non-inbred guinea-pigs used as controls in the Washington experiments and with other inbred families, family 2 has for generations been characterized by exceptionally light weight at all ages. During the period 1916 to 1924—the last period studied in detail (Wright and Eaton, '29)—it produced relatively small litters, but large numbers of litters per year, high percentage of young born

alive, and high percentage raised of those born alive. The family is noteworthy for its longevity, and has always been strikingly free from any tendency to produce abnormal young. The only abnormalities reported have been one or more cases of extra toes in the early generations prior to 1915 and a few cases of eye defects during the period covered by the present experiments, hereinafter referred to. The writer has never seen a club-foot, extra or missing toe, or other abnormality of the limbs, or a harelip or otocephalic condition in family 2, although he has seen all of these more or less frequently in other families—not all, however, in any one family—both inbreds and non-inbreds, raised under the same conditions during the course of these experiments.

Family 13, on the other hand, has for generations been characterized by relatively great weight at all ages (substantially equal to non-inbred stock). During the period 1916 to 1924 it produced larger litters and larger numbers of litters per year than family 2, but a lower percentage of young born alive and lower percentage raised of those born alive. On the whole, it has been a vigorous family. Besides the production in early generations of a few abnormalities of common types, it has had, in sharp contrast to family 2, a tendency to produce an exceptionally large percentage of otocephalic monsters, in some lines as high as 25.5 per cent, but about 4.0 per cent among some 1800 descendants of mating 13-20-3 of which the present data (showing 3.5 per cent) form a part. An extensive study of this type of hereditary abnormality, particularly in family 13, has been made by Wright and Eaton ('23). It had been shown by Wright ('22 a) that the tendency of a family to produce a given type of monster has no connection with the vigor of the family in other respects. Furthermore, he found no evidence that inbreeding has any specific causal connection with the origin of the monster. Inbreeding seemed merely to bring to light genetic traits already present in the original stock.

Care and feed

Since 1916 paired animals have been kept in metal pens about $16 \times 14 \times 24$ inches, with removable trays for ease in cleaning. The pens are stacked up in tiers, five or six pens high, and those containing the treated and control animals are scattered at random in order to minimize any slight differences in light, temperature, or other conditions. Effects due to seasonal changes have been controlled by making contemporaneous comparisons. The same care and feed have been given the animals in the present experiments as in the other animals in the colony. The feed is such as had been found from years of experience to keep the animals in health and reproductive vigor. The entire colony has been free from epidemics.

The data recorded

In practically all cases the litters have been recorded on the day on which they were born, occasionally not until the next day. A printed 'birth record' form is used for each litter, on which are entered the date of birth, pen no., mating no., and litter no., and for each pig, the sex, serial no., color, hair type, number of toes, and weight. The weight of sire and dam and abnormalities and other unusual conditions of parents or offspring are also recorded at this time. Drawings are made in a rubber-stamp outline, showing the color pattern of each pig. The colors, which for both family 2 and family 13 are black, red, and white spotted, in a highly variable pattern, make an almost certain means of identification of individuals. The ears are also punched as an aid in identification. The offspring are weighed a second time at thirty days of age and either mated at once or placed in stock pens.

In addition to the birth record, a double-entry card-index system for recording matings is used. There is one card for each experiment and another one for each pen. Still other facts such as weights of treated animals at the time of feeding lead, periodic weights of control animals, symptoms of

illness, results of autopsies, etc., have been recorded systematically.

The characters studied

Possible effects of lead have been looked for in fertility, abortions and stillbirths, birth weight, gain in weight, sex ratio, mortality during the first thirty days, and the production of abnormalities or mutations.

The writer considers himself most fortunate in having as experimental material pedigreed animals in which all these characters have been studied in detail for many generations.

Administration of lead

The lead has been administered in the form of neutral lead acetate ($Pb(CH_3COO)_2\,3H_2O$), weighed out on a chemical balance, placed in no. 4 gelatin capsules, and fed to the animal. No difficulty is encountered in pushing the capsule into the guinea-pig's throat with a finger. In most cases the capsule is accepted without a struggle and promptly swallowed. Treatment has been carried out either daily (six times a week) or on alternate days. The amount per dose has varied from 0.01 gram to 0.08 gram.

PRELIMINARY EXPERIMENTS

In July, 1926, preliminary experiments were begun, designed primarily to determine the dosage of lead suitable for producing symptoms of poisoning in guinea-pigs. Seven males were chosen from surplus stock, placed in individual breeding pens, and subjected to daily feeding with lead acetate. All seven were pedigreed animals, derived from several different experiments being carried on at that time by Doctor Wright.

Three of these males (♂'s 12, 27, and 28) came from family 13, hereinbefore described; ♂ 25535 came from family 2, also previously described; ♂ 89 came from a cross between a family-13 male and a family-2 female; and the other two (♂'s 31677 and 33) came from an experiment on coat color.

Six of the seven males were fairly young (from three months to eight months of age). They were, however, all old enough to be sexually mature. The sole criteria used in selecting the seven males for treatment were youth and health. Their weights at the time of beginning treatment are evidence of good condition, with the possible exception of ♂ 25535. His breeding history in the present experiments, however, shows nothing abnormal, and he survived intermittent treatment for seventeen months.

After treatment of the males had been continued for a month or more, two or three young females not previously mated were taken from stock and placed with each male. The females were an even more mixed group genetically than the males. Most of them came from the experiment on coat color referred to above; one female came from another experiment on coat and eye color; two were from family 2, one from family 13, and one from a cross between family 2 and family 13. Taken as a whole, these preliminary matings seem fairly well to meet the conditions of random matings, although an inspection of the source of the females leads one to expect offspring averaging lighter in weight than from matings between unrelated guinea-pigs. This is borne out by the results (table 1).

Effects of lead on the animals treated

The effects of lead on the weights of the animals are shown graphically in figures 3 and 4. There is no question but that treatment reduced the weights. It is also evident that the animals were able to survive daily ingestion of 0.01 gram or 0.02 gram of lead acetate daily for from one to two years, although all but one died prior to middle age. The maximum dose (0.08 gram) appeared to be clearly excessive.

Observations on the reproductive system indicated a tardy production of visible changes. Two of the males (♂ 's 31677 and 27) were examined after death for motile sperm and motile sperm were found. A testis from each was sectioned. That from the former was reduced in size; that from the lat-

ter was essentially normal in size. In both, spermatogenesis was under way. A testis from ♂ 12 was also sectioned. It showed some reduction in size, but spermatogenesis was under way. A unilateral castration was made of ♂ 25535 after two months' treatment at the maximum dosage used. Motile sperm were present. The testis was reduced in size, but spermatogenesis was under way.

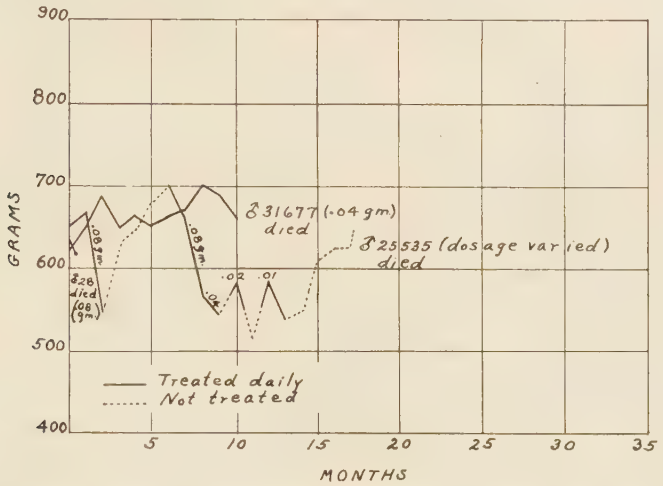

Fig. 3 Weight curves of three male guinea-pigs treated with lead acetate. Weights were taken daily. The weight entered on the graph for each month is the average for one week, using the monthly birthday as a center. Abrupt fluctuations due to the feeding of green feed, sometimes amounting to 50 grams or more, are thus minimized. Dosage is shown in grams.

One of the males (♂ 27) showed at autopsy the beginning of 'cirrhosis' at the edges of the liver, a condition which, as will be pointed out later herein, has been found consistently, in various degrees of severity, in animals from family 2.

Breeding results, the F1's

The breeding performance of the seven males is summarized in table 1. The problem of a critical external control with as heterogeneous a group of animals as this is an almost impossible one. The data are not submitted here as

critical evidence on the effect of lead on the offspring, but only for what light they may throw upon the results of the later experiments with inbred animals. In one sense, however, these experiments contain a control within themselves. Since lead is a cumulative poison whose visible pathological effects are brought about slowly after continued treatment, it is reasonable to suppose that if injury or alteration of the germ cells is effected, the most pronounced effects should

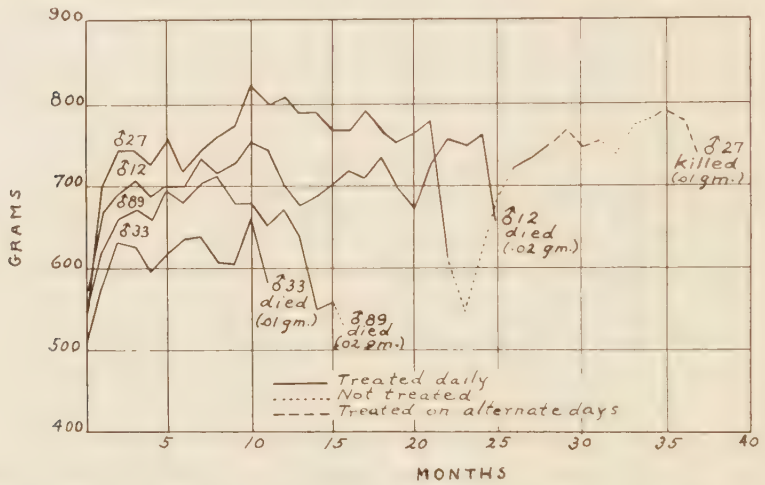

Fig. 4 Weight curves of four male guinea-pigs treated with lead acetate. Weights were taken daily. The weight entered on the graph for each month is the average for one week, using the monthly birthday as a center. Abrupt fluctuations due to the feeding of green feed, sometimes amounting to 50 grams or more, are thus minimized. Dosage is shown in grams.

come later. The litters produced by a given male after advanced treatment might be expected to show greater effects than the litters sired early. When the early litters were compared with the late ones, for each mating, no consistent differences as such a theory would require were found. Any slight differences existing might well be due to environmental conditions varying from season to season or from year to year, which have been shown by Wright ('22 a, '22 b) and by Wright and Eaton ('29) to produce decided effects on the offspring of guinea-pigs.

In size of litter, mortality percentages, birth weights, weights at thirty days of age, sex ratio, and the absence of abortions and abnormalities the results are good. On the face of the data there is nothing to indicate an effect of lead.

TABLE 1

Offspring from lead-treated males, preliminary experiments

Number of litters,	57	
Number of offspring,	146	
Average size of litter,	2.56	
Number born dead,[1]	22	(15.0 per cent)
Number died under 30 days of age,	16	(11.0 per cent)
Number raised to 30 days of age,	108	(74.0 per cent)
Average weight at birth of all those born,	71.2 grams	
Average weight at birth of all those raised,	75.3 grams	
Average weight at 30 days of age,	197.7 grams	
Sex ratio,	63 ♂ : 83 ♀	

[1] There were no cases of abortion or premature birth.

Effects in later generations, F2's, F3's, etc.

In view of the heterogeneous nature of the matings in the preliminary experiments, it was not considered desirable to make extensive matings between their offspring. Nevertheless, four such matings were made. There resulted 128 F2 offspring, all normal with the exception of four animals with eye defects. One had both eyes reduced in size, two had each a single eye reduced in size, and one was practically eyeless, having minute eye openings, but no eyeballs.

These defective individuals were all mated brother by sister or to parent, and in addition later to their offspring. There resulted thirty-four F3 offspring and fifty-four offspring from the back-cross matings. No abnormalities were observed with the exception of one otocephalic monster, like that found regularly in family 13, and one animal with a toe held slightly abnormally. The otocephalic monster was not unexpected, since as shown by his pedigree he was three-fourths blood family 13. A case of an otocephalic monster of three-fourths blood family 13 is reported by Wright and Eaton ('29).

In addition to the matings of defective individuals, other brother-sister F2 matings between the relatives of the defectives were made, resulting in sixty-nine F3 offspring, all normal.

In other respects the litters in the several generations seemed entirely typical. Does the appearance of the four defective-eyed animals indicate an effect of lead? Any dominant effect should have shown up in the F1's, but no such effect was found. Any recessive effect would most likely have shown up in the F3's, but none was found. The fact that the eye defects all appeared in the F2's would indicate, possibly, that a recessive tendency was present in the original matings. Three of the defective-eyed F2's trace back to an original mating between a family-13 male and hybrid female of quite a complex mixture whose hereditary tendencies are not fully known. Any recessive factor carried by her might of course be expected to show its effects in the F2's. On the other hand, any recessive effect induced in her treated mate would not be expected to appear until the F3 generation. The fourth defective-eyed F2 traces back to an original mating between a family-2 female and a family-13 male. As will be disclosed hereinafter, one case of persistent eye defect arose in the control line of family 2.

The fact that back-crosses and F2 matings failed to result in any abnormal offspring would seem to indicate that the eye defects might belong to that group of developmental irregularities having no demonstrable mendelian basis.

The defects were all of the type occurring sporadically in guinea-pigs. Doctor Wright states that he has observed in his experiments with untreated animals nine cases of absence of the eyeball on one or both sides during the time that the lead experiments have been under way. And Doctor Strandskov, in his laboratory, has also found two cases of absence of eyeballs (one unilateral and one bilateral) in a single litter of four from untreated animals.

EFFECTS OF LEAD ON INBRED MALES

Effects of lead on family-2 males

The experiments with family 2 were begun in March, 1927, and extended over a period of thirty-eight months, during which time forty-eight males were subjected to treatment. The symptoms of illness were numerous and severe and some were highly constant in occurrence. Loss in weight or failure to gain normally in weight was practically universal. The death rate was abnormally high. As one would naturally expect, the younger and lighter animals and those receiving larger or more frequent doses developed symptoms soonest; but when these variables are allowed for, there was remarkable uniformity of effect.

The animals ranged in age from one month up to and beyond middle age. Almost all of them, however, were quite young, the majority being less than two months of age and not yet sexually mature. The dosage used was in most cases 0.01 gram or 0.02 gram, but in a few cases with mature animals was 0.04 gram or 0.06 gram. In the latter cases the amount always had to be reduced before long, on account of the development of symptoms of illness presaging the death of the animal. In about half the cases treatment was daily (six times a week); in a number of others it was daily at the beginning and later changed to alternate days; and in the rest it was on alternate days from the start. With nearly half the animals, treatment was suspended for varying periods from one week up, on account of the development of severe symptoms; in most cases treatment was resumed.

Of the forty-eight males, thirty-five died and thirteen were killed—all but one of the latter at the conclusion of the experiment. Not one reached an advanced age. The animal reaching the greatest age died at three and one-half years; he had been under treatment for a period of two years and ten months, but for thirteen months of this time treatment was suspended because of various severe symptoms. The average age at death of the thirty-five animals was slightly less than

one and one-half years. In comparison with this there were during the same period of time thirty-nine untreated control males, and of these only six died. Their average age at death was two years eight months. One of them died at five years two months of age, siring a litter when past five years of age. As an indication of the natural longevity of family 2 there may be mentioned the recent death of a female at the age of six years seven months.

Of the forty-eight treated males, forty-two were autopsied: twenty-one (all but five of those treated for a year or more) showed a striking gross abnormality of the liver resembling human cirrhosis. This condition has been previously described by Ophüls ('15) in reporting experiments on the pathology of lead poisoning in guinea-pigs, in which lead carbonate was administered in the feed. No cases in the least resembling cirrhosis have been found in dozens of autopsies on untreated guinea-pigs in family 2 and other families.

It is worth noting that nearly all the more striking gross changes in the various organs described by Ophüls have been duplicated in the present experiments. For example, in sixteen of the animals with 'cirrhosis' there was a noticeable accumulation of more or less bloody fluid in the peritoneal cavity, ranging in amount from 37 grams up to 276 grams. Such fluid was also found in four cases in the absence of 'cirrhosis.'

Other pathological conditions noted less frequently were: fluid in the pleural cavities, seven cases; spleen enlarged, fibrous, and distorted, in most of the extreme cases; pericardium white and thickened, in the more advanced cases; unilateral eye infection, nine cases; edema, generalized, two cases, and localized in the throat region, two cases; extensive adhesions between the lungs and pleura, between the heart and pericardium, between the abdominal organs and the peritoneum or diaphragm, and between the organs themselves, found occasionally; typical lead anemia, as indicated by a somewhat reduced number of red corpuscles with an ab-

normally high resistance of the corpuscles to hemolysis in hypotonic salt solution, in the majority of the few cases tested.

In no case, however, was there observed any tendency of the treated animals to put on an excessive amount of fat as Ophüls found in some of his treated guinea-pigs, and without exception there appeared after extended treatment a marked loss in weight. In figure 5 are shown for comparison weight curves of treated males, untreated F1 males from treated

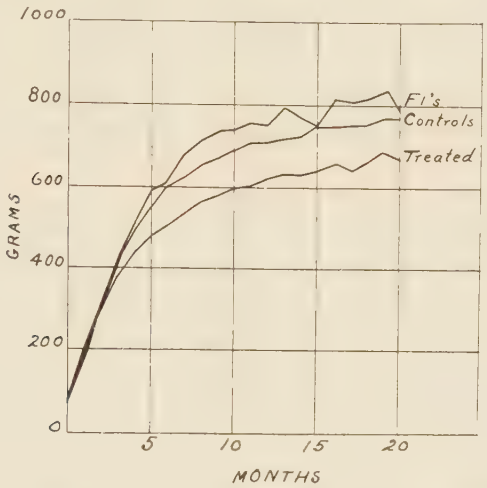

Fig. 5 Average weights of male guinea-pigs, family 2, showing the effect of lead in reducing the weight of treated males and the absence of such effect on the untreated offspring of treated males.

males, and control males. The treated animals were weighed regularly at the time of administration of lead. The F1's and controls were usually weighed weekly. The weights used for the treated males are averages for twenty-four males, being all those in which treatment was begun before three months of age and in which treatment was survived more than five months. The weights used for the F1's are averages for twenty males, being all those that were mated. The weights used for the controls are the averages for thirty-two males, including all control males with the exception of seven

that were adults when the experiments began and for which early weights by week were not available. The numbers averaged become less, however, as one moves to the right hand on the curves, on account of the death or slaughter or other disposition of certain of the animals, and in the case of the F1's also on account of the number of young animals still under twenty months of age.

With the controls, there were nineteen left to be weighed at the end of the tenth month and twelve at the end of the twentieth month. With the treated, there were twenty-one at the end of the tenth month and six at the end of the twentieth month. With the F1's, there were fourteen at the end of the tenth month and six at the end of the twentieth month. The small numbers account for the irregularities of the curves.

It will be noted that at birth as well as at one month of age the average weights of the three groups are almost identical. At one month of age the administration of lead is begun with nineteen of the twenty-four treated males. During the first month of treatment there is very little effect apparent, but during the second month of treatment there is a noticeable slowing down in rate of gain on the part of the treated males. The divergence in the curves increases for several months. After five months' treatment, the average difference between controls and treated remains at about 100 grams. As a matter of fact, the superiority of the controls would be still greater if deduction could be made for the artificial increase in weight of some of the treated animals due to the accumulation of fluid in the peritoneal cavity.

Aside from the foregoing objective evidence of the effect of lead on the weight of guinea-pigs, there is the strong impression gained in handling the animals daily of the poor condition and emaciation following long-continued treatment.

The curves indicate a slight superiority of the F1's over the controls. This difference can be interpreted otherwise, however, than as the effect of lead. In the first place, whereas the controls and treated are essentially contemporaneous, the

F1's come later in time, and there are some indications that conditions were more favorable for growth during this later time. In the second place, the average for the controls includes one animal that was unmistakably ill from about the third month until his death at fourteen and one-half months of age, apparently with tuberculosis. During this time he was regularly about 200 grams under the average weight. There were no such cases among the F1's. Near the end of the curves the small numbers involved make the differences unreliable.

The effect upon the reproductive system is, of course, of primary interest in connection with the breeding results. It was found to be very difficult to bring about extreme degeneration of the testis such as readily follows the application of heat and x-rays. In every one of the thirteen animals killed, eight of which had severe 'cirrhosis' of the liver, motile sperm were found in abundance in a suspension of material taken from the epididymis, although in three of these cases the testis was clearly reduced in size, in one markedly so.

A testis from one of the males killed earlier in the experiment (♂ 10698) was sectioned and studied histologically. Its size and appearance under the microscope are essentially normal. This animal had been treated on alternate days for seven and one-half months. His only symptoms noted were a severe eye infection and some emaciation.

In seven of the animals that died similar examinations for motile sperm were made, and in all but two of them motile sperm were found. The two exceptions were males 166 and 329, which had been under treatment for two years ten months and two years one month, respectively, and at death showed extreme pathological conditions. Male 166 had been dead for some time when found; no sperm, dead or alive, could be discovered. The testis, which was very small, was not sectioned. Male 329 was observed at death; no motile sperm could be seen. A testis was preserved and sectioned. On the slide it measures slightly more than half the diameter of the normal testis. The tubules are small and closely packed and contain in most cases a single layer of cells.

Another one of the seven (♂ 10404) was also observed at death. He had been under treatment for one year eight and one-half months and showed severe symptoms, including extreme 'cirrhosis,' ascites, edema, eye infection, and lung infection. Examination disclosed many motile sperm. The testis measures about half the diameter of the normal testis. Viewed in section, it resembles that of ♂ 329, except that in some of the tubules several layers of cells are present.

The seminal vesicles in the extreme cases of lead poisoning were commonly reduced in diameter, shortened, and knobby in outline.

In concluding the consideration of the pathological effects of lead on males in family 2, it may be stated that although it was found relatively easy to induce a variety of severe symptoms leading to early death, by feeding lead acetate daily or on alternate days, it was very difficult to produce absolute sterility in the sense that no sperm were developed. It is, of course, conceivable that sperm may be injured or altered without the destruction of the capacity for motility and fertilization. This possibility, which the experiment was designed to test, will be considered hereinafter in an analysis of the breeding results.

Effects of lead on family-13 males

The effects of treatment on three family-13 males in the preliminary experiments have already been described. It may be recalled that one of these three showed at death the early stages of 'cirrhosis.' Three additional family-13 males were subsequently treated and mated to family-13 females exclusively. Weight curves of these three are shown in figure 6. Effects on weight similar to those in family 2 are here indicated. All three were killed at the conclusion of the experiment. No gross abnormalities were found. In each case the testis appeared normal, and motile sperm in abundance were observed.

There was some indication that family 13 was more resistant to the poisonous effects of lead than family 2, although

the small number of males from family 13 used for treatment and the fact that four of the six were killed, perhaps too soon for treatment to have produced obvious effects, make this by no means certain.

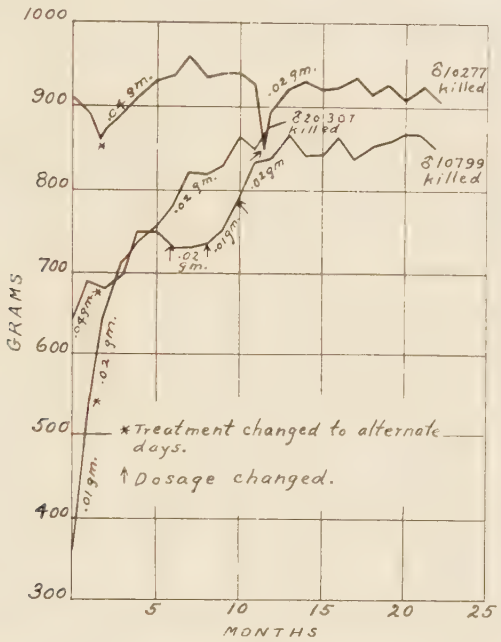

Fig. 6 Weight curves of three male guinea-pigs, family 13, treated with lead acetate. Weights were taken at the time of administration of lead. The weight entered on the graph for each month is the average for one week, using the monthly birthday as a center. Abrupt fluctuations due to the feeding of green feed, sometimes amounting to 50 grams or more, are thus minimized. Dosage is shown in grams.

BREEDING METHODS, FAMILY 2 AND FAMILY 13

Family 2

The origin and previous history of the inbred families are given under Material and Methods (p. 439). In March, 1927, the matings on hand in family 2 were divided into two equal lots, in one of which the males alone were treated with lead, and in the other no treatment was administered. As new matings were made additional males were subjected to treat-

ment. It was the aim throughout to keep on hand as nearly as possible equal numbers of control and treated matings. At the beginning of the experiment there was a single pair of animals in each pen. In the present experiments in a number of cases in both control and treated lines two females were mated to one male in the same pen. In all such cases the females were sisters of the male, though not always litter mates.

Since the period of gestation in guinea-pigs averages about sixty-eight days, the first litters entered as offspring from treated males were born at least this long after treatment of the sire was begun. Five matings produced litters, first, as controls and later, after the male had been treated. Most of the treated males came from untreated parents; treatment was therefore for only one generation. Seven of the treated males, however, came from treated sires. The period of treatment in such cases was not long, varying from one month up to four months prior to conception. These seven males, mated to their sisters, really constitute F1 matings, but they have not been so tabulated, since it was desired to count them as P1 matings and it was not desired to count them twice.

The offspring raised from the treated males, but themselves untreated, were mated brother to sister and designated as F1 matings. Twenty-three such F1 matings made up of twenty-three females mated to eighteen males have produced offspring. Offspring raised from the F1 matings were mated brother to sister and designated as F2 matings. Twenty-two such F2 matings made up of twenty-two females mated to sixteen males have produced offspring.

A group of non-brother-sister matings was also made, four males being treated and mated to surplus family-2 females without regard to relationship. All but one of these males were from control matings; the one exception came from a sire that had been treated for four months prior to conception. Each male was placed in a floor pen, 24 × 36 inches, with ten or eleven females. As the females were found to be

pregnant, usually at five or six weeks, they were removed to the small breeding pens, usually two pregnant females to a pen, and left there until their litters were born and raised, after which they were returned to the floor pen. The females came from control matings. The purpose of the arrangement in this series was to test the effects of lead when there was eliminated so far as practicable the possibility of the females and offspring getting lead from the contamination of food and water by the urine or feces of the treated males. Such a possibility is, of course, always present when the dam and young remain with the treated male throughout. Due to lack of space, no similar control series of floor pens was run.

Family 13

Although the experiments were largely confined to family 2, similar experiments on a smaller scale were done with family 13. Two of three family-13 males treated in the preliminary experiments lived for some time, and after their mates in the preliminary experiments had died, they were mated to family-13 females not their sisters. Three additional family-13 males were later treated and mated to family-13 females without regard to relationship. One of these males (♂ 10277) was placed in a floor pen like that mentioned above with about a dozen females. The other males were mated in small breeding pens, two females in a pen. All the females used in this series with the exception of two were from untreated brother-sister matings; two females were daughters of treated ♂ 12. All the straight brother-sister matings in family 13, as shown in the pedigree chart (fig. 2), were kept as controls.

BREEDING RESULTS, FAMILY 2 AND FAMILY 13

Sterility

At one extreme in the possible effect on fertility is the production of absolute sterility. The term 'sterility' is used in this connection for want of a better one, since in no case has it been determined that failure to produce offspring was

the result of failure to produce germ cells rather than merely failure to copulate. Out of twenty-seven treated family-2 males that lived to be one year of age, only two failed to produce offspring within the first year. One of these was killed at sixteen months of age, following fifteen months' treatment. The testes were of normal size, and motile sperm in large numbers were observed. At two months of age his first mate died and at five months of age two other females were placed with him. Neither ever became pregnant, so far as could be detected. The other male was killed at eighteen months of age, following seventeen months' treatment. The testes were of normal size, and motile sperm in large numbers were observed. From the eleventh month on, his mate was examined on alternate days; she never became pregnant, so far as could be detected. A second family-2 female placed with him during the last four months likewise failed to become pregnant. In order to test for sterility in the first female, another male known to be fertile was placed in the pen after the former male was killed. A normal litter resulted.

There have been no cases like the above among the controls in the present experiments, and no cases have been previously reported for family 2. A few cases in other families, however, are mentioned by Wright ('22 b). Nevertheless, certain of the control matings have failed to produce litters over long periods of time.

In family 13 there have been no cases, either among the treated or control animals, of failure to produce offspring within the first year, and none has been previously recorded.

Size and frequency of litter

As shown in table 2, the controls and treated, brother-sister matings, family 2, had litters closely comparable in size. In contrast to this, the treated males in floor pens, non-brother-sister, and the F1's and F2's from treated sires, but themselves untreated, produced much larger litters. The superiority of the F1's and F2's might be taken to indicate a selective effect. However, a large part of the difference is

TABLE 2

Data on the fertility of control and treated lines, families 2 and 13

EXPERIMENT	NUMBER OF LITTERS OF SPECIFIED SIZE						TOTAL LITTERS	NUMBER YOUNG BORN	AVERAGE SIZE OF LITTER	AVERAGE INTERVAL BETWEEN LITTERS, MONTHS
	Bi-maternal	1	2	3	4	5				
Family 2										
Brother-sister matings:										
June, 1927, to April, 1930										
Controls, 1♀ in a pen	..	16	47	36	6	1	106	247	2.33	3.1
Controls, 2♀'s in a pen	2	5	25	13	2	.	47	108	2.30	2.9
Total controls	2	21	72	49	8	1	153	355	2.32	3.0
June, 1927, to April, 1930										
♂'s treated, 1♀ in a pen	..	6	29	16	9	1	61	153	2.51	3.3
♂'s treated, 2♀'s in a pen	2	7	36	22	4	.	71	165	2.32	3.0
Total ♂'s treated	2	13	65	38	13	1	132	318	2.41	3.1
January, 1929, to May, 1930										
Untreated from treated:										
F1 matings, 1♀ in a pen	..	4	11	19	12	.	46	131	2.85	2.9
F1 matings, 2♀'s in a pen	2	3	4	9	6	.	24	68	2.83	3.0
Total F1 matings	2	7	15	28	18	.	70	199	2.84	2.9
June, 1929, to June, 1930										
2nd untreated generation:										
F2 matings, 1♀ in a pen	..	1	6	7	3	1	18	51	2.83	2.5
F2 matings, 2♀'s in a pen	4	2	5	5	2	1	19	53	2.79	4.0
Total F2 matings	4	3	11	12	5	2	37	104	2.81	3.1
Non-brother-sister matings:										
September, 1928, to May, 1930										
♂'s treated, floor pens	8	3	15	32	12	.	70	203	2.90	4.3
Grand total, family 2	18	47	178	159	56	4	462	1179	2.55	..
Family 13										
Brother-sister matings:										
June, 1928, to May, 1930										
Controls, 1♀ in a pen	..	17	33	50	17	.	117	301	2.57	3.5
Non-brother-sister matings:										
June, 1928, to May, 1930										
♂'s[1] treated, 2♀'s in a pen	..	5	10	10	5	1	31	80	2.58	3.9
November, 1928, to May, 1930										
♂10277 treated, floor pen	..	4	7	17	13	.	41	121	2.95	4.5
Total ♂'s treated	..	9	17	27	18	1	72	201	2.79	4.3
January,[2] 1929, to April, 1930										
Untreated-treated line:										
F1[3] matings, 1 and 2 ♀'s in a pen	..	1	10	12	2	1	26	70	2.69	3.0
F2[3] matings, 2♀'s in a pen	..	2	7	6	4	.	19	50	2.63	3.0
Grand total, family 13	..	29	67	95	41	2	234	622	2.66	..

[1] Males moved from pen to pen for a part of the time.
[2] Four scattered litters from F1's were born previous to this period.
[3] Matings brother × sister, although the P1 matings were not.

undoubtedly due to lack of complete simultaneousness in the experiments. The controls and treated, brother-sister, were begun earlier than the others, and there was found among the controls a significant increase in litter size during the latter part of the experiment (1930 average, 2.63; 1928 average, 2.24; probability, 0.04). Nevertheless, even when the two groups are compared over the same period of time, a significant difference remains; the difference between the F1's and F2's combined and all the controls (sixty-one litters) over the same time is $2.83 - 2.50$, or $0.33 \pm .14$. The difference is 2.34 times its standard error, which means a probability of its occurring by chance about twice in one hundred times. In view of this result, it was thought interesting to retain the F1 and F2 and the control matings for a while longer, to see whether the difference would persist or not. The matings were therefore kept up to the end of October, 1930. Including this last period, the average for the F1's and F2's was 2.77 (153 litters) and for the controls, 2.54 (seventy-three litters); difference, $0.23 \pm .12$; probability, 0.06.

The difference between the averages of the treated, non-brother-sister matings and the controls over the same period of time (seventy-nine litters) is $0.59 \pm .14$; probability, about 0.00001. Are we to conclude that this highly significant difference is due to treatment of the sire? A more probable explanation seems to be the long interval between litters in the non-brother-sister matings. As previously explained, the females in these matings were mated in floor pens and isolated from the treated males as soon as found to be pregnant and until after the weaning of their litters at thirty days of age. This isolation necessarily increased the interval between litters. As shown in table 2, the average interval was actually about one month longer for these matings than for the controls. Likewise in family 13, in which there was also a group of matings in which the females were isolated, the difference is in the same direction as in family 2, though not so great, 2.95 for treated and 2.61 for controls (ninety-six litters) over

the same period of time, making a difference of $0.34 \pm .17$; probability, 0.045. As tending to support this explanation are data published by Wright ('22 a) for much larger numbers of litters. He found that for various inbred families, combined, the average size of litter was 2.22 where the litter immediately succeeded another (interval merely the gestation period) and 2.46 after a longer interval. Moreover, the fact that no significant increase occurred in the treated, brother-sister matings, family 2, in which the interval was the same as in the controls argues against lead as the causative agent of the large litters in the non-brother-sister matings.

Summing up, although it is difficult to explain fully the large litters in the F1's and F2's, it seems doubtful that lead was a factor. There certainly is no evidence of a deleterious effect of lead.

As to frequency of litter, little need be said except that there is no difference between the control and treated lines in matings where male and female were together throughout. In the great majority of cases the treated males continued to produce litters quite regularly close up to the time of death, suggesting that the germ cells were able to resist the effects of the poison longer than the cells of certain vital organs of the body. This is perhaps what one would anticipate from the finding of motile sperm in the treated animals at death.

Abortions and stillbirths

The percentages born dead in each of the experiments with family 2 and family 13 are shown in table 3. Is there any indication in any of these percentages of an effect of lead? In determining the standard error of a percentage the formula used is $\text{S.E.}\, q = 100\sqrt{pq/n}$, where $p + q = 1$ and $n =$ the number of cases. This formula can be correctly applied only where the n cases are entirely independent of one another. Obviously, in guinea-pigs where multiple births are the rule, abortions and stillbirths are not independent of one another. In more than 50 per cent of animals born dead in family 2 the entire litter is born dead, the animals being distinctly

TABLE 3

The percentage of young born dead, the percentage that died prior to thirty days of age, the percentage raised to thirty days of age, the average birth weight of all young born, the average birth weight of young raised to thirty days of age, and the average weight at thirty days of age, families 2 and 13[1]

EXPERIMENT	BORN DEAD	DIED	RAISED	AVERAGE BIRTH WEIGHT, ALL	CORRECTED AVERAGE	AVERAGE BIRTH WEIGHT, RAISED	CORRECTED AVERAGE	AVERAGE WEIGHT, 30 DAYS	CORRECTED AVERAGE
Family 2									
Brother-sister matings:	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Controls, 1♀ in a pen	24.1	8.0	67.9	68.7	67.1	75.1	73.4	187.0	183.4
Controls, 2♀'s in a pen	26.0	21.2	52.9	68.1	65.9	74.1	72.0	178.5	174.4
Total controls	24.6	11.9	63.5	68.6	66.8	75.0	73.1	185.0	181.1
♂'s treated, 1♀ in a pen	24.8	20.3	54.9	67.1	67.1	72.9	73.0	177.8	178.0
♂'s treated, 2♀'s in a pen	18.6	27.5	53.9	69.6	67.8	74.7	72.8	171.5	167.9
Total ♂'s treated	21.6	24.1	54.4	68.5	67.7	73.8	72.9	174.5	172.8
F1 matings, 1♀ in a pen	20.6	21.4	58.0	71.2	74.8	74.1	77.8	194.9	202.9
F1 matings, 2♀'s in a pen	25.0	22.1	52.9	66.2	69.3	71.8	75.2	169.1	175.7
Total F1 matings	22.1	21.6	56.3	69.4	72.7	73.3	76.8	186.5	194.0
F2 matings, 1♀ in a pen	29.4	17.6	52.9	64.1	67.1	69.2	72.5	182.1	189.2
F2 matings, 2♀'s in a pen	32.1	41.5	26.4	59.3	61.7	71.1	74.0	171.5	177.3
Total F2 matings	30.8	29.8	39.4	61.7	64.3	69.8	72.9	176.1	182.4
Non-brother-sister matings:									
♂'s treated, floor pens	32.0	19.2	48.8	63.3	66.9	70.9	74.9	184.9	193.6
Family 13									
Brother-sister matings:									
Controls, 1♀ in a pen	12.3	13.6	74.1	81.7	82.5	83.6	84.3	241.4	243.3
Non-brother-sister matings:									
♂'s treated, 2♀'s in a pen	25.0	10.0	65.0	76.0	76.8	81.4	82.3	249.3	251.5
♂10277 treated, floor pen	21.5	20.7	57.9	75.7	80.5	83.1	88.4	234.4	246.6
Total ♂'s treated	22.9	16.4	60.7	75.8	78.9	82.3	85.7	240.8	249.0
F1 matings, 1 and 2♀'s in a pen	21.4	7.1	71.4	81.6	83.8	85.4	86.3	268.8	271.0
F2 matings, 2♀'s in a pen	30.0	28.0	42.0	84.7	86.2	90.6	90.6	269.0	271.6

[1] Data on the six characters considered in this table have been tabulated separately for each size of litter and are presented in the doctor's thesis by the author ('30), on file at The University of Chicago.

small and premature. The litter in such cases is therefore really the independent event. If n in the above formula is taken as the number of offspring, the $\sqrt{n}$ is found to be about 50 per cent larger than n taken as the number of litters. The true n must lie somewhere in between. Consequently, the method used by Wright ('22 b) has been adopted. This consists in striking a compromise, using n as the number of offspring in calculating the standard error, then increasing the standard error by 25 per cent to allow for the correlation between litter mates. The standard error of the difference between the two percentages to be compared is the square root of the sum of the squares of the two standard errors. These methods have been applied to the data on percentage born dead as well as to the data on percentage that died and percentage raised.

If we regard a probability of 0.05 or less as indicating a significant difference, there are in family 2 no differences between the controls and treated lines that approach significance. In family 13, however, the controls seem to be superior to the treated lines (controls, 12.3 per cent; all treated lines combined, 23.7 per cent; probability, 0.003). This, of course, disregards such important factors as number of females in a pen, seasonal variations, and size of litter. Moreover, it seems that the controls in family 13 have recently been producing an abnormally small percentage of dead-born. The percentage 12.3 per cent does not agree at all well with the past history of family 13. During each of the years 1916 to 1924—the last period studied in detail—the family produced more than 21 per cent of dead-born (Wright and Eaton, '29). The average for the nine years was 26.6 per cent of 2765 born. This is seen to be higher than the percentage in the treated lines in the present experiments.

The results, therefore, in the experiment as a whole do not seem to point convincingly toward an injurious prenatal effect of lead, and certainly indicate no such marked effects as those reported in man by Paul and by Deneufbourg.

Postnatal mortality

The data on percentage that died prior to weaning are summarized in table 3. Several quite large differences are apparent in family 2. Between the controls and treated, one female in a pen, the difference is 12.3 ± 4.60 per cent; probability, 0.008. With two females in a pen, the difference is in the same direction, though not significant. When the two groups are combined, the difference becomes 12.2 ± 3.64 per cent; probability, 0.001. There is in these figures a distinct suggestion of an effect of lead, but this may be through absorption of lead by the young from food and water contaminated by the urine and feces of the sire. It is also likely that difference in size of litter may account for the difference in part. In tabulations of mortality percentages according to size of litter, it was found in all cases that young produced in large litters, especially litters of four or more, are at a distinct disadvantage. A little experience with guinea-pigs demonstrates that animals in small litters are distinctly larger and more vigorous than those in large litters. Just how much should be allowed for difference in size of litter cannot be accurately determined, and no correction factor has been introduced herein.

When we compare the mortality in the controls with that in the F1's and F2's and non-brother-sister matings in family 2, the factor of size of litter becomes especially important, since in the latter three groups the litter size is appreciably larger than in the controls. Neglecting this factor and making a comparison over the same period of time, the largest difference was between the controls and the F1's, one female in a pen; probability, 0.006.

On the other hand, in family 13 there are no differences that are statistically significant. Considering both families together, therefore, the evidence does not seem convincing that treatment of the sire has had an effect on the postnatal mortality of the young.

The percentage raised of all young born

In some respects the percentage raised is a more desirable character for study than either the percentage born dead or the percentage died. Some of those found and recorded as dead must in reality have died after birth and so should be counted as died. It is also quite possible that certain factors that tend to cause stillbirths would, if slightly less intense, tend to cause postnatal mortality. Thus an unusually low prenatal mortality might be compensated for by a high postnatal mortality, and vice versa.

The data on percentage raised are summarized in table 3. As to the effect of an extra female in a pen, it is interesting to note that in every case a poorer showing is made in pens with two females; not all the differences, however, are statistically significant.

As between the controls and treated lines in family 2, there are several significant differences. With the controls and treated, one female in a pen, the difference is 13 per cent; probability, 0.04; with two females in a pen the treated are slightly superior, so that the difference between the total controls and total treated becomes 9.1 per cent; probability, 0.055. Both groups of F1's and F2's, one female in a pen, are slightly, though not significantly, superior to the controls over the same period of time. The F2's, two females in a pen, are poorer than the controls: difference, 26.5 per cent; probability, 0.05. The larger litters of the F2's may account for this difference. With the treated non-brother-sister matings and the total controls over the same period of time, the difference is 17 per cent in favor of the controls; probability, 0.006. The much larger litters of the treated may account for this difference.

In family 13 there are two significant differences, both in favor of the controls. With the controls and treated ♂ 10277 the difference is 15.0 per cent; probability, 0.025; with the controls and the F2's the difference is 29.2 per cent; probability, 0.002. The differences may be due to difference in size of litter and the presence of an extra female in the pen in the case of treated lines.

It appears, therefore, that the controls, though actually better than the treated lines in percentage raised, are not necessarily so as a result of treatment, but perhaps largely because of a difference in litter size and possibly also as a result of some absorption of lead by the offspring themselves. There is also the possibility that the females with treated males may have absorbed enough lead in the food or water contaminated by the males to have affected the mortality of the young. Observations of the living females and autopsies, however, gave no evidence of lead poisoning in the females.

Birth weights and weights at thirty days of age

Guinea-pigs vary greatly in weight at birth and at thirty days of age, due to a variety of factors, environmental and hereditary. Among the environmental factors, size of litter is perhaps the most important. Animals born in small litters are much heavier than those born in large litters. Those born in litters of one, for example, are more than 50 per cent heavier than those born in litters of four. There are various methods of correcting for this factor when different experiments are to be compared. One method is to compare litters of the same size only; but this has the disadvantage that the numbers in an ordinary experiment become small when thus broken up. Nor does the method consider the experiment as a whole, which is what one is most interested in. A single corrected figure for litters of all sizes combined is therefore desirable.

A method for obtaining such a figure is described by Wright and Eaton ('29). It was found that the regressions of birth weights as well as weights at thirty-three days of age on litter size were not strictly linear, since a difference of one in size of litter made more difference in weight in small litters than in large litters, resulting in curves concave above. The regressions were also found to be greater in heavy families such as family 13 than in light ones such as family 2. It was shown that the use of logarithms of the weights tended to

straighten out the curves and make them parallel. The average regressions of the logarithms of the birth weights of young raised and of weights at thirty-three days were calculated for large numbers of animals. From these averages tables of factors were prepared which, when multiplied by the actual averages, reduced the weights to a standard litter size of 2.5.

In the present experiments the average regressions of the birth weights of all young born, of the young raised, and of the weights at thirty days in family 2 and family 13 have been calculated and found to agree closely with those obtained in the study just referred to. The same correction factors have therefore been used and the figures appearing in table 3 under 'corrected average' are the products of such factors and the actual averages.

Variations in weights are also dependent more or less on seasonal and annual changes. This factor has been minimized herein by comparing the treated and control lines over the same periods of time. It is still possible that slight seasonal effects are left uncorrected, due to the fact that it is not practicable to breed guinea-pigs in such a way that litters will be produced in different experiments strictly contemporaneously throughout the year. This cannot be very important, however, for in most of the experiments the litters are pretty well scattered over the period covered thereby.

There is also some variation dependent on sex. Males average slightly heavier at birth and at thirty days of age than females. Since in most of the experiments the ratio of males to females has been close to 1:1, this factor can be of little weight. The two sexes are therefore tabulated together.

One other correction that needs to be made relates to the correlations between litter mates. It is obvious that such correlations must exist, since the environmental factors that affect the weights will in general act alike upon litter mates. Consequently, the individual weights in multiple births are not independent events, and the true n used in calculating the standard errors must lie somewhere between the number

of animals and the number of litters, just as in the case of mortality percentages. Again following the method used by Wright in previous studies of these families, the standard errors have been calculated, using n as the number of animals, and the standard errors have then been increased 25 per cent to allow for correlation between litter mates.

TABLE 4

Differences between the average weights of the control and treated lines and of the controls of family 13 and family 2, with the probability that each difference might be exceeded in either direction by random sampling

	BIRTH WEIGHTS ALL BORN		BIRTH WEIGHTS RAISED		30-DAY WEIGHTS	
	Difference	Probability	Difference	Probability	Difference	Probability
Family 2						
Controls, 1 ♀—Pb, 1 ♀	0.0	1.00	+ 0.4	.86	+ 5.4	.42
Controls, 2 ♀'s—Pb, 2 ♀'s	— 1.9	.45	— 0.8	.78	+ 6.5	.48
Controls, total—F1's, total	— 2.4	.14	+ 3.0	.21	+ 11.4	.13
Controls, total—F2's, total	+ 2.3	.54	+ 2.1	.60	+ 14.6	.17
Controls, total—Pb, non-brother-sister	+ 2.3	.39	+ 2.5	.27	+ 9.6	.20
Family 13						
Controls, 1 ♀—Pb, 2 ♀'s	+ 5.7	.07	+ 1.1	.72	— 12.9	.27
Controls, 1 ♀—Pb, ♂10277	+ 4.2	.07	— 1.9	.49	+ 3.2	.78
Controls, 1 ♀—F1's, 1 ♀ + 2 ♀'s	+ 1.0	.75	+ 0.9	.77	— 18.1	.09
Controls, 1 ♀—F2's, 2 ♀'s	— 1.4	.66	— 5.87	.22	— 6.7	.68
Family 13, G1 ♀—family 2, G1 ♀	+ 12.8	.0000001	+ 10.9	.0000001	+ 47.1	.000000001

In this study detailed comparisons have been made under two classifications: 1) the birth weights of all those born, dead or alive; 2) the birth weights of all those raised. The average birth weights and thirty-day weights are summarized in table 3. The differences between the averages, corrected for size of litter according to the method just described, with the probability that each difference might be exceeded in either direction by random sampling, are shown in table 4.

Standard deviations for birth weights of those raised run as follows: family 2, about 14 grams; family 13, about 17.5 grams. For all those born, dead or alive, it is also about 17.5 grams in family 13, but about 20 grams in family 2, due to the relatively large number of small aborted litters in family 2. Standard deviations for weights at thirty days of age run as follows: family 2, about 40 grams; family 13, about 65 grams.

As between the controls and treated lines there is obviously not one significant difference in either family. In startling contrast is the highly significant difference between the controls of family 2 and family 13, where the only differential factors are hereditary ones.

Everything considered, therefore, we find no evidence that lead administered to the sire has any effect upon the birth weight or weight at thirty days of age of the offspring. Nor is there any evidence that the offspring of treated males fail to gain normally in weight throughout the growth period. The curve showing average weights for F1 males up to twenty months of age (fig. 5) is slightly above that for the controls.

Sex ratio

Experiments in the Bureau of Animal Industry at Washington have shown a sex ratio for guinea-pigs close to 1:1, with a slight advantage in favor of males. For example, among the controls produced for the years 1906 to 1920 there were 2051 males and 2007 females. Among the inbreds for the same period there were 12,831 males and 12,529 females (Wright, '22 a).

In the present experiments the sex ratio has likewise been close to 1:1. As shown in table 5, there are in family 2 no differences that reach significance, taking a probability of 0.05 or less as an indication of significance. Family 13 as a whole, however, shows a significant excess of males. No significant differences from a 1:1 ratio have been reported heretofore for either family. During the years 1916 to 1919 family 13 produced a slight excess of females and family 2

a larger excess of males—just the reverse of the situation in the present experiments. Excluding the F2 matings, the difference in family 13, as shown in table 5, is not significant (probability, 0.18). The abnormal ratio in the F2's resulted

TABLE 5

Sex of young produced in the different experiments, with the probability that each difference from a 1:1 ratio might be exceeded in either direction by random sampling. (Those of unknown sex were found dead and either partly eaten or in bad condition)

EXPERIMENT	MALES	FEMALES	SEX UNKNOWN	TOTAL	PER CENT MALES OF KNOWN SEX	PER CENT FEMALES OF KNOWN SEX	PROBABILITY
Family 2							
Brother-sister matings:							
Controls, 1 ♀ in a pen	108	134	5	247	44.6	55.4	.10
Controls, 2 ♀'s in a pen	51	47	10	108	52.0	48.0	.69
Total controls	159	181	15	355	46.8	53.2	.23
♂'s treated, 1 ♀ in a pen	78	69	6	153	53.1	46.9	.46
♂'s treated, 2 ♀'s in a pen	79	79	7	165	50.0	50.0	1.00
Total ♂'s treated	157	148	13	318	51.5	48.5	.61
F1 matings, 1 ♀ in a pen	69	57	5	131	54.8	45.2	.29
F1 matings, 2 ♀'s in a pen	36	30	2	68	54.5	45.5	.46
Total F1 matings	105	87	7	199	54.7	45.3	.20
F2 matings, 1 ♀ in a pen	18	27	6	51	40.0	60.0	.18
F2 matings, 2 ♀'s in a pen	29	21	3	53	58.0	42.0	.26
Total F2 matings	47	48	9	104	49.5	50.5	.90
Non-brother-sister matings:							
♂'s treated, floor pens	91	107	5	203	46.0	54.0	.25
Total, family 2	559	571	49	1179	49.5	50.5	.71
Family 13							
Brother-sister matings:							
Controls, 1 ♀ in a pen	150	144	7	301	51.0	49.0	.75
Non-brother-sister matings:							
♂'s treated, 2 ♀'s in a pen	43	36	1	80	54.4	45.6	.43
♂ 10277 treated, floor pen	68	53	0	121	56.2	43.8	.17
Total ♂'s treated	111	89	1	201	55.5	44.5	.12
F1 matings, 1 and 2 ♀'s in a pen	37	33	..	70	52.9	47.1	.63
F2 matings, 2 ♀'s in a pen	34	15	1	50	69.4	30.6	.007[1]
Total, family 13	332	281	9	622	54.2	45.8	.04

[1] With the addition of five recent litters mentioned in the text, the ratio is 40 males to 22 females (probability, 0.02).

from the performance of one male mated to two of his sisters. With one of the sisters he produced eleven males and two females and with the other, twelve males and two females—total, twenty-three males and four females (probability, 0.0003). It seemed interesting to retain these two matings after the experiment as a whole was discontinued. Up to the present time (May, 1931) they have produced five additional litters, consisting of six males and seven females, making the ratio now twenty-nine males and eleven females (probability, 0.004). That the excess of males in family 13 is due to the administration of lead seems open to doubt, in view of the negative results in family 2 and in the preliminary experiments.

Abnormalities and possible mutations

As mentioned under Material and Methods, family 2 has always been strikingly free from any tendency to produce abnormal young. In family 13, on the other hand, there early became established a hereditary tendency to produce a relatively high percentage of 'otocephalic' monsters. In both family 2 and family 13 there has been observed not infrequently a certain type of white opacity of the lens in newborn young. The condition is visible on close examination with the naked eye. With a magnifying glass, the lens appears to be divided into three or four segments, the lines bounding the segments meeting in the center and forming a star-shaped figure. It is noted more often in weak or dead animals than in others. In case the animal lives, the condition clears up in a day or so. It has also been observed to appear in a few cases after birth in animals apparently normal at birth. Such animals always died. There has been no discoverable connection between the condition and lead poisoning.

Aside from the foregoing, the only abnormalities observed in family 2 have been three cases of eye defects, one in the controls, one in the F1's, and one in an experiment with treated females. The first case mentioned (♀11624) was

produced by mating 2-23-12 C in their sixth and last litter. The other members of the litter, a male and a female, were apparently normal. The defective-eyed female was above the average in weight (80 grams) and the sire and dam both seemed in good condition. The entire litter was raised. The abnormality involved the right eye only. The eyeball was much smaller than the left and there was no iris or pupil, the white sclera extending over the surface, which was marked by irregular spots and streaks of brown pigment. The eye opening was only about 5 mm. long, instead of 8 mm. as in the left eye.

At weaning the female was left in the pen with her sire. By him she produced two litters; the first consisted of three normal animals, all of which were raised; the second consisted of two, both of which were weak and died the first day. When found, they both had white opaque lenses like those temporary ones referred to above. At the birth of this last litter the dam was in poor condition; her hair was thin and she was light in weight.

The defective-eyed female was then mated to her son. After eight months, she produced one litter and this was aborted early and eaten before being found. A few days later, she died.

The second animal with defective eyes (♂ 12140) was produced by mating F1 2-22-32. It was born dead, September, 1929, in a litter of one as the fifth litter. It was a large, well-nourished animal weighing 90 grams. The sire and dam were apparently in good condition. The defect consisted of the absence of the right eyeball. The eye opening was about half the length of the left. The left eye was apparently normal. No further litters were recorded from this pair until eleven months later, when an early aborted litter was found. The sire of the abnormal male was born after thirteen months' treatment of the grandsire and its dam after six and one-half months' treatment of the grandsire.

In family 13, abnormalities, aside from otocephalic monsters, were no more frequent than in family 2. Among the

controls there occurred the only limb abnormalities observed in the present experiments. In June, 1929, mating 13-29-8 produced as their first litter two offsprings, a male and a female, both of which were abnormal. The fore feet of the male were bent inward, so that he walked on the backs of the feet. The toes were flexed. The hind legs were abnormally short. He died at five days of age. His litter mate had the same type of abnormality of the front limbs. The hind legs were very short and an extra toe was hanging from the lateral side of each hind foot. She died at nine days of age. A second litter produced by the same pair was apparently normal.

In the treated line of family 13 there was one case of eye abnormality. Male 27 used in the preliminary experiments was the sire. The dam was a family-13 female not his sister. In their second litter, born February, 1929, there were two males, in one of which, ♂ 11525, the left eye seemed smaller than the right and the lids were held nearly closed. Otherwise both offspring seemed normal. The dam appeared in good condition. When examined three days after birth, the eye appeared 'milky' and small; there was some discharge. At ten days after birth the eye was thought to be infected. When the animal was weaned at thirty days of age, the lids were stuck shut and the eyeball seemed smaller than the right.

He was mated to his sister born in the previous litter. They produced four litters, including thirteen offspring. No eye defects were found, but in the third litter, consisting of three males and one female, there was one male with a small blister-like object in the center of the forehead. This soon dried up, forming a small scab about 2 mm. in diameter, which was still present when he was weaned at thirty days of age.

Male 11525 was also back-crossed to two of his daughters. Each of these matings produced three litters, with a total of fourteen offspring. All were normal with the exception of one typical otocephalic monster.

The otocephalic monsters produced by family 13 are as follows:

	Males	*Females*	*Sex unknown*	*Total*	*Per cent*
Controls,	5	4	1	10	3.32
Total males treated,	4	3	0	7	3.48
F1 matings,	0	4	0	4	5.71
F2 matings,	1	0	0	1	2.00

In no case is the percentage produced by the treated lines significantly different from the controls. In fact, the percentages for the controls and the treated males are closer than we have any right to expect. The others are what would be expected from random sampling.

In conclusion, it appears that there is no significant difference in abnormalities produced by control and treated lines in any of the experiments. Those few abnormal offspring that lived were mated and produced offspring without any evidence appearing that they were the result of mutations. The most likely interpretation is that they belong to that group of developmental irregularities due to such causes as nutritional disturbances of the dam.

TREATMENT OF FEMALES, FAMILY 2

The evidence from clinical studies and from animal experimentation makes it seem quite certain that lead poisoning of the female may induce abortion or stillbirth in man as well as in other mammals (Hamilton, '25, and Aub et al., '26). Although not strictly a part of the present problem, it seemed interesting to try out on a small scale the effects of lead administered to females from family 2. Accordingly, five females were treated and mated to untreated family-2 males, two females in a pen. The females were all well within the reproductive age. Four of them had previously produced normal living litters in other matings; one had not been previously mated. Treatment in each case was begun with 0.01 gram administered daily. In two cases it was stopped outright after two months, because of a marked loss in weight, and not resumed; in the other three cases treatment was changed after a time to administration on alternate days.

The females apparently reacted to the treatment in the same way as males. The two that received lead for the

longest time (twenty-one and sixteen months, respectively) developed the same 'cirrhosis' of the liver as males, accompanied in each case by an accumulation of fluid in the peritoneal cavity. The one that was treated twenty-one months contained 372 grams of fluid, a relatively enormous amount in comparison with the weight of the body (682 grams) after its removal.

To the five treated females only three living litters were born during the whole time. Three of the females produced no living litters, one produced one living litter, and one produced two living litters. Four aborted litters were found in the pens. In eight other instances positive diagnosis of pregnancy was made by palpation, followed later by signs of abortion, but without the discovery of the embryos themselves. With guinea-pigs the products of an early abortion are almost invariably eaten unless discovered promptly, and although each female was examined carefully either daily or on alternate days, it was not surprising that a majority of the early aborted litters were not found. One of the females was in the early stages of pregnancy at death.

A word should be said about the two living litters produced by the female mentioned above. Contrary to all the other cases, treatment was begun with her while she was already in the advanced stages of pregnancy. The first litter was born at the end of twenty-four days' treatment. This litter, consisting of two males and one female, seemed perfectly normal. One of the males died on the second day after birth. The other male and the female were mated at thirty days of age in order to test for recessive effects. This mating produced five litters, consisting of seventeen offspring (three dead, four died, ten raised). None was premature and all were apparently normal.

One F2 mating was made between litter mates born in the second litter. This mating produced five litters, consisting of sixteen offspring (one dead, four died, eleven raised). None was premature and all were normal with the exception of one female, born in the last litter, in July, 1930. At birth

her right lens was opaque. The opacity was of a different type from the temporary opacity previously described. It persisted with little change, and at weaning time the female was left in the pen to mate with her sire. By him she produced one litter of three, all normal. No other F2 matings were made.

The second litter produced by the treated female just considered was composed of five males, all born alive and all apparently normal. Three died and two were raised.

The female that produced a single living litter had aborted four months previously. She had been treated for nine months prior to the birth of the living litter. This litter consisted of two females, both apparently normal. Both died.

Taken as a whole, the breeding results of the treated females present a bad record as compared to controls, and tend to confirm the generally held opinion as to the tendency of lead to induce abortions. The five females were mated for a total of more than seven years during the reproductive period, and, as described above, only three full-term litters were born. There is, however, a lack of consistency among the histories, and it is difficult to explain the three living litters if we regard the abortions as due to lead. It is certainly true that no five females could be selected from the controls with as bad a recorded history of abortions as the five treated females; but this is not a fair comparison to make, since the control females were not examined regularly as were the treated females. It is known that early abortions sometimes occur among the controls, and, as previously stated, if the embryos are very small, they are entirely eaten. There were several control females that produced a few litters and then stopped producing for many months. We have no means of knowing how many abortions may have occurred during those months. It seems, therefore, that all we can say in conclusion is that there is a good indication of the effect of lead in producing abortions; there is no evidence of any effect on the few offspring that were born alive. Without further evidence it would be unsafe to attribute the single eye defect among the F3's to treatment.

DISCUSSION

Since the results in the present experiments have been largely negative so far as effects upon the offspring are concerned, it may be useful at this point to compare them with other similar experiments wherein positive effects have been claimed. In their experiments with rabbits, Cole and Bachhuber studied two characters, mortality percentages and birth weights. The mortality percentages were designated as per cent dead in four days. The results indicated a higher mortality rate and lower birth weights as a result of treatment. The statistical significance of the differences between the controls and treated lines is not discussed.

The formula for testing the significance of a percentage difference previously used herein is very simply applied to their data presented herein on page 431. When this is done, the difference in mortality percentages in series I proves to be clearly significant. No allowance is made, however, for possible correlation between litter mates nor for difference in size of litter. And if rabbits are at all like guinea-pigs in this respect, there is certainly some correlation between litter mates in mortality rate and between litter size and mortality rate. If such correlations were taken into consideration, it might turn out that the difference in series I is not significant after all. Without making any corrections, the difference in series II is clearly not significant.

With regard to the birth weights, the significance of the differences cannot be determined from the averages alone. No mention is made of the size of litter, and in rabbits as well as in guinea-pigs there appears to be a negative correlation between birth weight and size of litter (Kopeć, '24). This factor may therefore account for the differences found. At any rate, with numbers no larger than these one would wish to know the standard errors of the means before accepting the differences between them as significant.

Another question that arises relates to the genetic constitution of the rabbits used. What great effects on weight may result from genetic differences in guinea-pigs have been

confirmed in the present experiments. Even though the two albino rabbits used were of equal weight, it is still possible that a difference in genetic constitution may have influenced the result. Furthermore, it is not stated whether the same females were used in series I and in series II nor whether those used in treated and control lines were homogeneous genetically. The results of the system of double matings do not furnish a complete control on the female side, since less than half of the matings were successful as double matings.

In the case of fowls the chicks from poisoned White Leghorn cocks showed in every case a higher prenatal as well as postnatal mortality than those from the untreated Houdan cock. But here again there is a possibility of unknown genetic differences to have entered with the sperm. It is conceivable that the differences between the offspring of the Houdan cock and the White Leghorn cock may have been the result of hybrid vigor rather than lead.

The results in Weller's experiments with guinea-pigs differ sharply from the present experiments in that a distinct reduction in birth weight was found to follow treatment in the former. In the present experiments there was no significant effect of lead on birth weights. The differences in weight found by Weller are comparable to the differences between family 13 and family 2, but no statistical tests are applied to his data, and the significance of the differences cannot be determined from the averages alone. Provided the differences are significant, it is still possible that they may be due to genetic differences.

Weller found no differences in numbers of stillborn between the controls and treated males. With regard to the mortality during the first week, the difference, although in favor of the controls, is clearly not significant, even though no allowance is made for correlations between litter mates and between mortality rates and litter size. The difference in percentage is 2.07 ± 2.8; probability, 0.46.

The author states (but without presenting figures) that there is a retardation in development such that the offspring of a lead-poisoned male remain permanently underweight. As shown in a preceding section, no such effect has been found in the present experiments.

There is agreement between the two experiments in the lack of any trustworthy evidence of the production of abnormalities or mutations.

In view, therefore, of all the evidence in this and other experiments, it is yet to be demonstrated that lead administered to the sire has any effect upon the offspring of mammals.

SUMMARY AND CONCLUSIONS

1. In a series of experiments carried on continuously over a period of four years, fifty-eight male guinea-pigs and five female guinea-pigs were fed weighed amounts of lead acetate. All but four of the animals came from two closely inbred lines, families 2 and 13, derived from single pairs in the fifteenth and twentieth generations, respectively, of straight brother-sister mating. The individuals within each family were remarkably homogeneous in all respects studied.

2. The symptoms of lead poisoning were numerous and severe, and some, e.g., 'cirrhosis' of the liver, were highly constant in occurrence. The life span was materially shortened. In most of the males that died or that were killed motile sperm were found. In only a few cases was marked degeneration of the testis evident from histological studies.

3. The inbred males receiving lead were mated to untreated females of the same inbred families, in most cases brother to sister, and the resulting 722 offspring compared with the 656 offspring from untreated brother-sister matings of the same family. The offspring from the treated sires were mated brother to sister and the resulting 269 F2's compared with the controls. The F2's were also mated and the resulting 154 F3's compared with the controls.

4. As concerns sterility, there were only two males from family 2 that lived to be twelve months of age without pro-

ducing offspring. Both were treated males. They were killed at sixteen and eighteen months of age, respectively, without ever having produced offspring or even signs of pregnancy. The testes of both were of normal size, and large numbers of motile sperm were present. No similar cases were observed in family 13. In almost all cases the treated males in both families continued to produce offspring with normal regularity close up to the time of death.

5. The evidence indicated that lead administered to males had little or no effect on size or frequency of litter. The average litter produced by treated males, non-brother-sister matings, and by untreated F1's and F2's was larger than the controls. In the case of the non-brother-sister matings, the difference seems due in part at least to increased interval between litters. With the F1's and F2's the difference is not so easily explained, although it seems doubtful that the difference was due to lead.

6. In a few instances there were significant differences between the controls and treated males in percentage of offspring born dead or dying within thirty days of age, but when allowance is made for difference in size of litter and the possibility of the offspring getting lead from contaminated food or water, the evidence that the differences were due to treatment of the sire is not convincing.

7. There were no significant differences between the controls and treated lines in birth weights nor in weights at thirty days of age. In contrast to this, there were highly significant differences in weights between the controls of family 2 and family 13, where the only differential factors were hereditary ones.

8. The only significant departure from a sex ratio of 1:1 was in family 13. In family 13 as a whole there was an excess of males, with a probability of its being exceeded in either direction by random sampling of 0.04. Upon analysis, this appears to be due largely, if not entirely, to the highly abnormal ratios produced by two matings among the F2's. Negative results in family 2 and the preliminary experiments cast doubt upon the suggestion that lead was responsible.

9. With family 13 the normal percentage of otocephalic monsters was produced, with no significant difference between the controls and treated lines.

10. A few other abnormalities were observed, chiefly eye defects of one kind or another. The frequency of these was no greater among the treated lines than among the controls. Breeding results failed to reveal any of these abnormalities as hereditary.

11. With the administration of lead to females from family 2 there were good indications of an increase in the frequency of abortions. There was no evidence of any effect on the few living offspring that were produced.

LITERATURE CITED

AUB, J. C., FAIRHALL, L. T., MINOT, A. S., AND REZNIKOFF, P. 1926 Lead poisoning. Baltimore: Williams & Wilkins Co.

COCKAYNE, E. A. 1927 Melanic examples of Selenia bilunaria. Proc. Ent. Soc. Lond., vol. 2, pp. 23–24.

COLE, L. J., AND BACHHUBER, L. J. 1914 The effect of lead on the germ cells of the male rabbit and fowl as indicated by their progeny. Proc. Soc. Exp. Biol., vol. 12, pp. 24–29.

DENEUFBOURG, H. 1905 L'intoxication saturnine dans ses rapports avec la grossesse. Thèse de Paris.

FISH, H. D. 1914 On the progressive increase of homozygosis in brother-sister matings. Amer. Nat., vol. 48, pp. 759–761.

HAMILTON, ALICE 1925 Industrial poisons in the United States. New York: The Macmillan Co.

HARRISON, J. W. H. 1927 A further induction of melanism in the Lepidopterous insect, Selenia bilunaria Esp., and its inheritance. Proc. Royal Soc., B, vol. 102, pp. 338–347.

HARRISON, J. W. H., AND GARRETT, F. C. 1925 The induction of melanism in the Lepidoptera and its subsequent inheritance. Proc. Royal Soc., B, vol. 99, pp. 241–263.

KOPEĆ, STEFAN 1924 On the influence exerted by certain inheritance factors on the birth weight of rabbits. Anat. Rec., vol. 27, pp. 95–118.

LEGGE, THOMAS M., AND GOADBY, KENNETH W. 1912 Lead poisoning and lead absorption. London and New York.

LOEB, L., AND WRIGHT, S. 1927 Transplantation and individuality differentials in inbred families of guinea-pigs. Amer. Jour. Path., vol. 3, pp. 251–283.

MANN, MARGARET C. 1923 A demonstration of the stability of the genes of an inbred stock of Drosophila melanogaster under experimental conditions. Jour. Exp. Zoöl., vol. 38, pp. 213–244.

MANSBRIDGE, W. 1927 Examples of melanism in Lepidoptera. Proc. Ent. Soc. Lond., vol. 2, pp. 20–22.

MULLER, H. J. 1928 The production of mutations by x-rays. Proc. Nat. Acad. Sci., vol. 14, pp. 714–726.

NEWMAN, H. H. 1921 The experimental production of twins and double monsters in larvae of the starfish Patiria miniata, together with a discussion of the causes of twinning in general. Jour. Exp. Zoöl., vol. 33, pp. 321–352.

OLIVER, T. 1911 A lecture on lead poisoning and the race. Brit. M. J., vol. 1, p. 1096.

OPHÜLS, W. 1915 Chronic lead-poisoning in guinea-pigs: with special reference to nephritis, cirrhosis, and polyserositis. Am. Jour. Med. Sci., vol. 150, pp. 518–541.

PAUL, CONSTANTIN 1860 Étude sur l'intoxication lente par les préparations de plomb, de son influence sur le produit de la conception. Arch. Gen. de Med., T. 1 (5th series, vol. 15), pp. 513–533.

PEARL, R. 1914 On the results of inbreeding a mendelian population; a correction and extension of previous conclusions. Amer. Nat., vol. 48, pp. 57–62.

RENNERT, O. 1881 Ueber eine hereditäre Folge der chronischen Bleivergiftung. Arch. f. Gyn., Berlin, Bd. 18, S. 109–131.

SONNEBORN, T. M. 1930 Genetic studies in Stenostomum incaudatum. II. The effects of lead acetate on the hereditary constitution. Jour. Exp. Zoöl., vol. 57, pp. 409–439.

WELLER, C. V. 1915 The blastophthoric effect of chronic lead poisoning. Jour. Med. Res., vol. 33, pp. 271–293.

———— 1916 Histological study of the testes of guinea pigs showing lead blastophthoria. Preliminary report. Proc. Soc. Exp. Biol. and Med., vol. 14, p. 14.

WRIGHT, S. 1922 a The effects of inbreeding and crossbreeding on guinea pigs. I. Decline of vigor. II. Differentiation among inbred families. U. S. Dept. Agric. Bull. no. 1090.

———— 1922 b The effects of inbreeding and crossbreeding on guinea pigs. III. Crosses between highly inbred families. U. S. Dept. Agric. Bull. no. 1121.

———— 1931 Evolution in mendelian populations. Genetics, vol. 16, pp. 97–159.

WRIGHT, S., AND EATON, O. N. 1923 Factors which determine otocephaly in guinea pigs. Jour. Agric. Res., vol. 26, pp. 161–181.

———— 1929 The persistence of differentiation among inbred families of guinea pigs. U. S. Dept. of Agric. Tech. Bull. no. 103.